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Catechol-*O*-methyltransferase mRNA in the kidney and its appearance during ontogeny

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Catechol-*O*-methyltransferase mRNA in the kidney and its appearance during ontogeny. Catechol-*O*-methyltransferase (COMT), primarily present as a soluble cytosolic form (S-COMT), inactivates catechols. The recent cloning of the rat and human S-COMT from placenta has allowed us to synthesize complementary oligonucleotide probes to study the localization of COMT mRNA during development in the rat kidney and in the adult human kidney using *in situ* hybridization histochemistry. In the adult rat kidney, COMT mRNA was detected in segment S3 of proximal tubule cells in the outer stripe of the outer medulla, and thick ascending limb of loop of Henle (TAL) in the inner stripe. COMT mRNA was detected in the prenatal rat kidney as early as on day 18. In the human kidney, strong hybridization signal was seen in the medulla and in tubule segments of the cortex. In the adult rat kidney, COMT mRNA was in addition demonstrated in the transitional epithelium of the ureter. The results suggest synthesis of COMT and inactivation of catechols along the distal parts of proximal tubules, in TAL cells, and in the epithelium of the ureter.

The enzyme catechol-*O*-methyltransferase (COMT; E.C. 2.1.1.6.) catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to the *m*-hydroxy group of various catechols [1–3]. The *O*-methylation reaction is important in the enzymatic inactivation of catecholamines released from catecholaminergic neurons [4, 5] or circulating in plasma [6, 7], enzymatic inactivation of 2-hydroxy-estrogens [8], or the detoxification of xenobiotic catechols [9]. At least two isoforms of COMT exist, of which a soluble form (S-COMT) predominates, while there are smaller amounts of membrane-bound enzyme (MB-COMT) [10, 11], the latter having a higher affinity for its catechol substrates [12, 13].

COMT activity is widely distributed in various tissues including, for example, brain [14, 15], liver [16], placenta [17], gut [18], heart [19], uterus [20] and kidney [14, 21]. In the kidney, COMT has been demonstrated by immunohistochemistry in the luminal surface of proximal and distal convoluted tubules and in collecting ducts [14].

Catecholamines play an important role for the regulation of renal function, particularly for the regulation of renal sodium excretion. All catecholamines are freely filtered and the kidney is responsible for at least 15% of the overall plasma clearance of catecholamines [see 22]. Dopamine is synthesized in proximal tubule cells and the concentration of dopamine in renal tissue is much higher than the concentration of dopamine in the blood. Catecholamines, filtered, or synthesized in the kidney, will be excreted in the urine either in native form or as metabolites. Since catecholamine receptors have been identified in the apical membrane of renal tubule cells [23], native catecholamines in the tubular fluid may regulate the function of tubular cells. Thus, it is important to localize the catecholamine-metabolizing enzymes in the kidney.

Recently, the cDNAs for rat and human S-COMT [24, 25] and human MB-COMT [26] have been cloned and characterized. In this study oligonucleotide probes complementary to rat and human S-COMT mRNA were synthesized and used for *in situ* hybridization histochemistry on sections from rat and human kidney. The presence and distribution of COMT mRNA was examined in the kidney during pre- and postnatal development.

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Methods

In situ *hybridization*

Kidneys from Sprague-Dawley rats at prenatal days 18 and 21, within 24 hours of birth, postnatal days 8, 10, 20 and 40, were dissected out, frozen on dry ice, sectioned at 14 μm in a cryostat (Dittes, Heidelberg, Germany), and thaw-mounted onto precleaned ProbeOn™ microscope slides (Fisher Scientific, Pittsburgh, Pennsylvania, USA). A human kidney was obtained from a nephrectomized patient due to a hypernephroma tumor. Two 48-mer oligonucleotide probes were synthesized complementary to nucleotides 87-130 of rat [24] and nucleotides 467-504 of human [25] COMT mRNA. The probes were 3′-end labeled with ^{35}S-dATP (NEN, Boston, Massachusetts, USA) using terminal deoxynucleotidyl transferase (Amersham Ltd., Amersham, UK) and purified using Nensorb 20 columns (NEN). *In situ* hybridization was performed essentially as described [27, 28]. In brief, the tissue sections were air-dried and incubated for 16 hours at 42°C with 10^6 cpm of the labeled probe in a hybridization solution containing 50% deionized formamide (Baker Chemicals, Deventer, The Netherlands), SSC in 4 × SSC concentration (1 × SSC = 0.15 M NaCl, 0.015 M sodium-citrate), 1 × Denhardt's solution [0.02% bovine serum albumin, 0.02% Ficoll (Pharmacia, Uppsala, Sweden), 0.02% polyvinylpyrolidone], 1% sarcosyl, 0.02 M $NaPO_4$ (pH 7.0), 10% dextran sulphate (pH 7.0) (Pharmacia), 500 mg/ml denaturated salmon sperm DNA (Sigma, St. Louis, Missouri, USA) and 200 mM dithiothreitol (LKB, Stockholm, Sweden). After hybridization (16 hours), the sections were

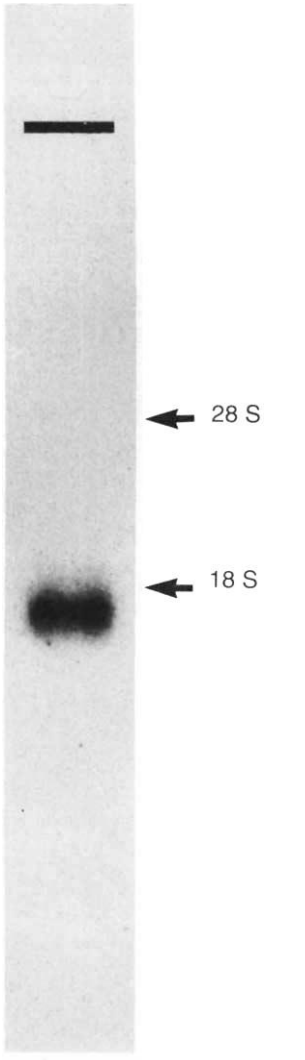


Fig. 1. *Northern blot analysis of total RNA extracted from whole adult rat kidney after hybridization with an oligonucleotide probe to rat COMT mRNA.* A single transcript of approximately 1.8 kb in length is observed. The positions of the 28S and 18S ribosomal bands are indicated.

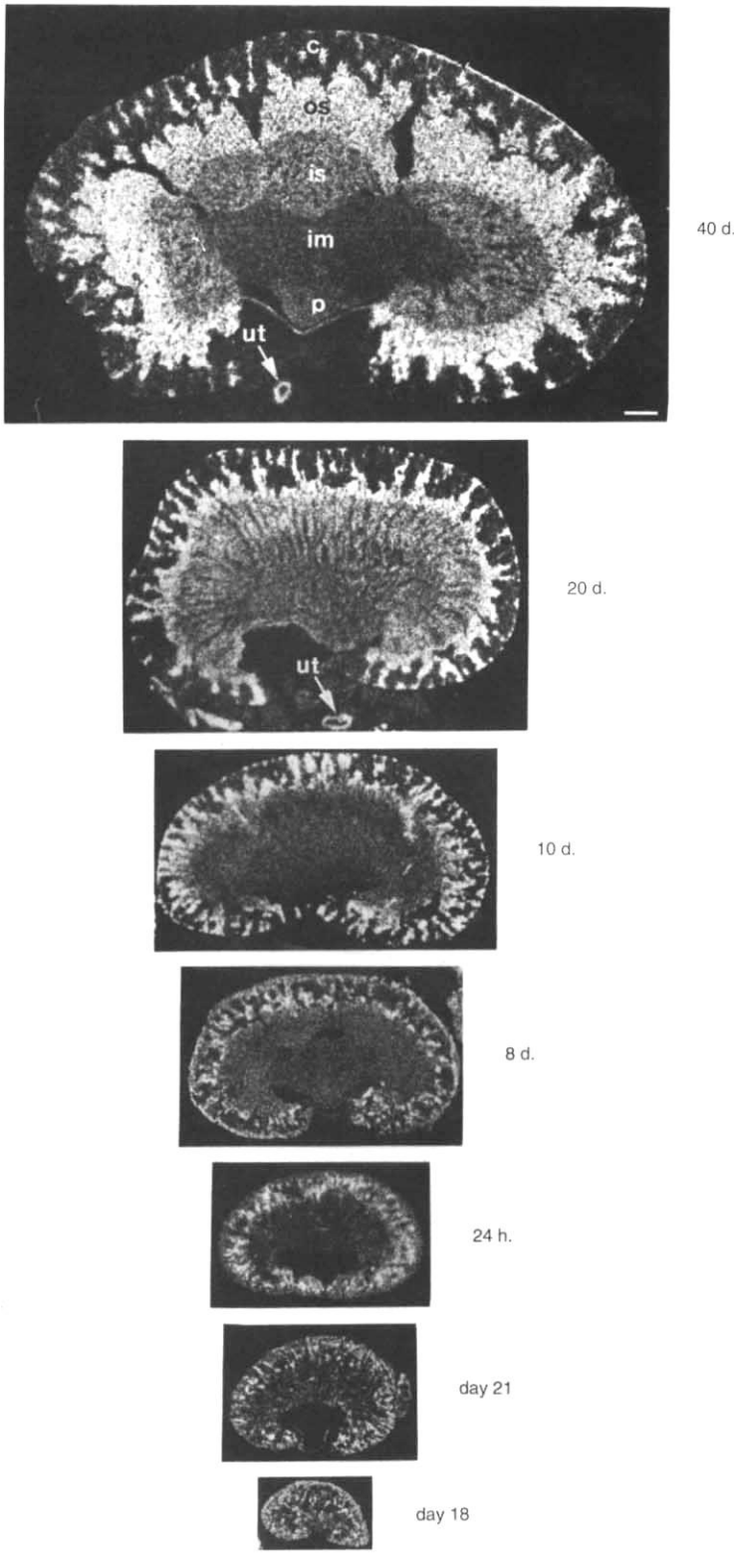


Fig. 2. *Film autoradiograms of sections of rat kidney at postnatal days 40, 20, 10 and 8, within 24 hours of birth, and prenatal days 18 and 21 after* in situ *hybridization with an oligonucleotide probe to COMT mRNA.* In the adult kidney, strong hybridization signal is distributed in the outer stripe (os) of the outer medulla, with rays radiating into the cortex (c). Weaker labeling is present in the inner stripe (is) of the outer medulla and also in the papilla (p). Virtually no labeling is present in the outer cortex or in the inner medulla (im). Note intense labeling in the ureter (ut; arrow) in 40 and 20 day old kidneys. COMT mRNA is apparent in the kidney as early as on prenatal day 18. The strong labeling in the os is apparent from postnatal day 8. During prenatal day 18 to 10 days postnatally, the COMT mRNA labeling is diffusely distributed in the cortex. At 20 days there is a more organized pattern of labeling, with a pattern resembling that seen at 40 days. Bar = 1 mm.

rinsed in 1 × SSC at 55°C for 15 minutes with four changes of SSC and for 60 minutes at room temperature, transferred through distilled water, dehydrated through 60% and 95% ethanol (two minutes each) and apposed to Hyperfilm β-max autoradiography film (Amersham) at −20°C. After 14 days of exposure, the film was developed with Kodak LX 24 for four

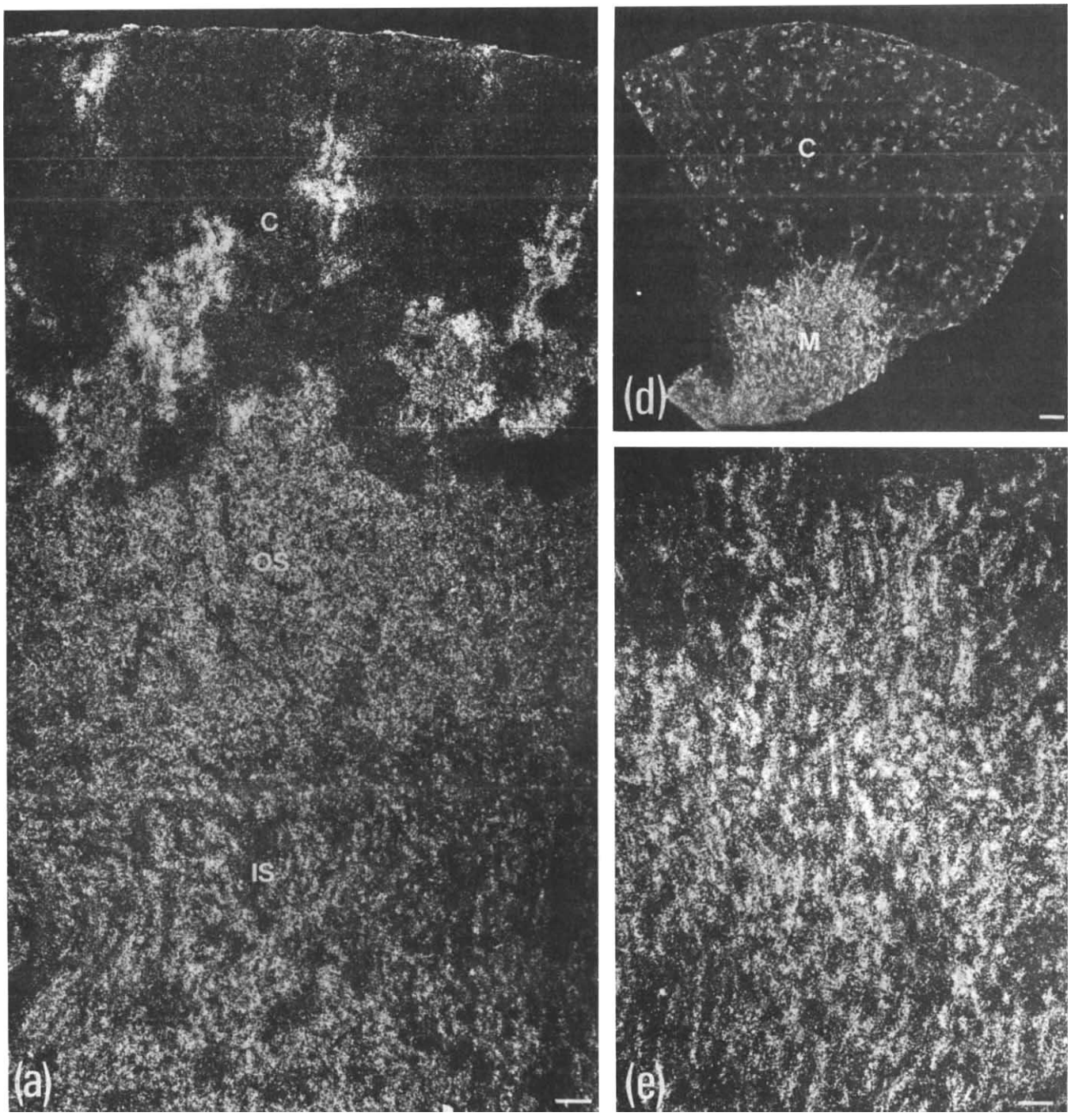
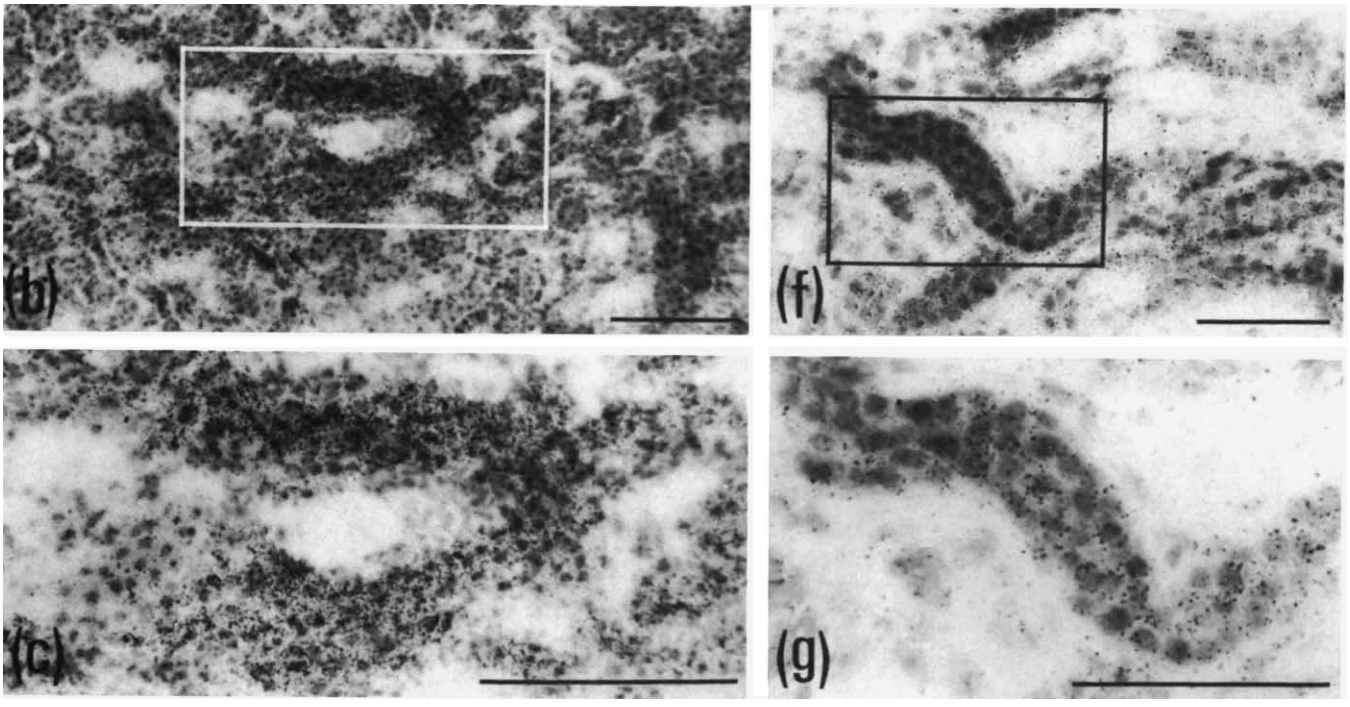


Fig. 3. *Film autoradiogram (d), dark- (a,e) and bright-field (b,c,f,g) photomicrographs of emulsion-dipped sections of adult rat (a-c) and human (d-f) kidneys after* in situ *hybridization with oligonucleotide probes to COMT mRNA.* In rat kidney, labeling is observed in the outer stripe (OS) and in rays radiating into the cortex (C), and in nephron segments in the inner stripe (IS). In the human kidney, labeling is seen in scattered tubular structures in the entire cortex (C) and in the medulla (M) (Fig. 3d). After counter-staining with hematoxylin-eosin, it can be seen that silver grains are overlying proximal tubule cells located in the OS in rat (b; higher magnification in c), and in TAL cells in the M of human (e; higher magnification in f) kidney. Bar in a-c = 50 μm, e and f = 100 μm, and in d = 1 mm. (*Continued on next page*)

minutes and fixed for 15 minutes with Kodak AL 4. Some sections were dipped in Kodak NTB_2 emulsion, exposed for five weeks at 20°C, and developed in Kodak D19 (4 min) and fixed in Kodak 3000 (10 min). The slides were rinsed in distilled water and coverslipped with glycerol. In addition, some of these sections were counter-stained with hematoxylin-eosin, dehydrated in graded series of ethanol and coverslipped with Entellan® (Merck, Darmstadt, Germany). All sections were



examined in a Nikon Microphot-FX microscope equipped for bright-field and dark-field microscopy. Photographs were taken with Kodak Tmax 100 ASA black and white film.

Northern analysis

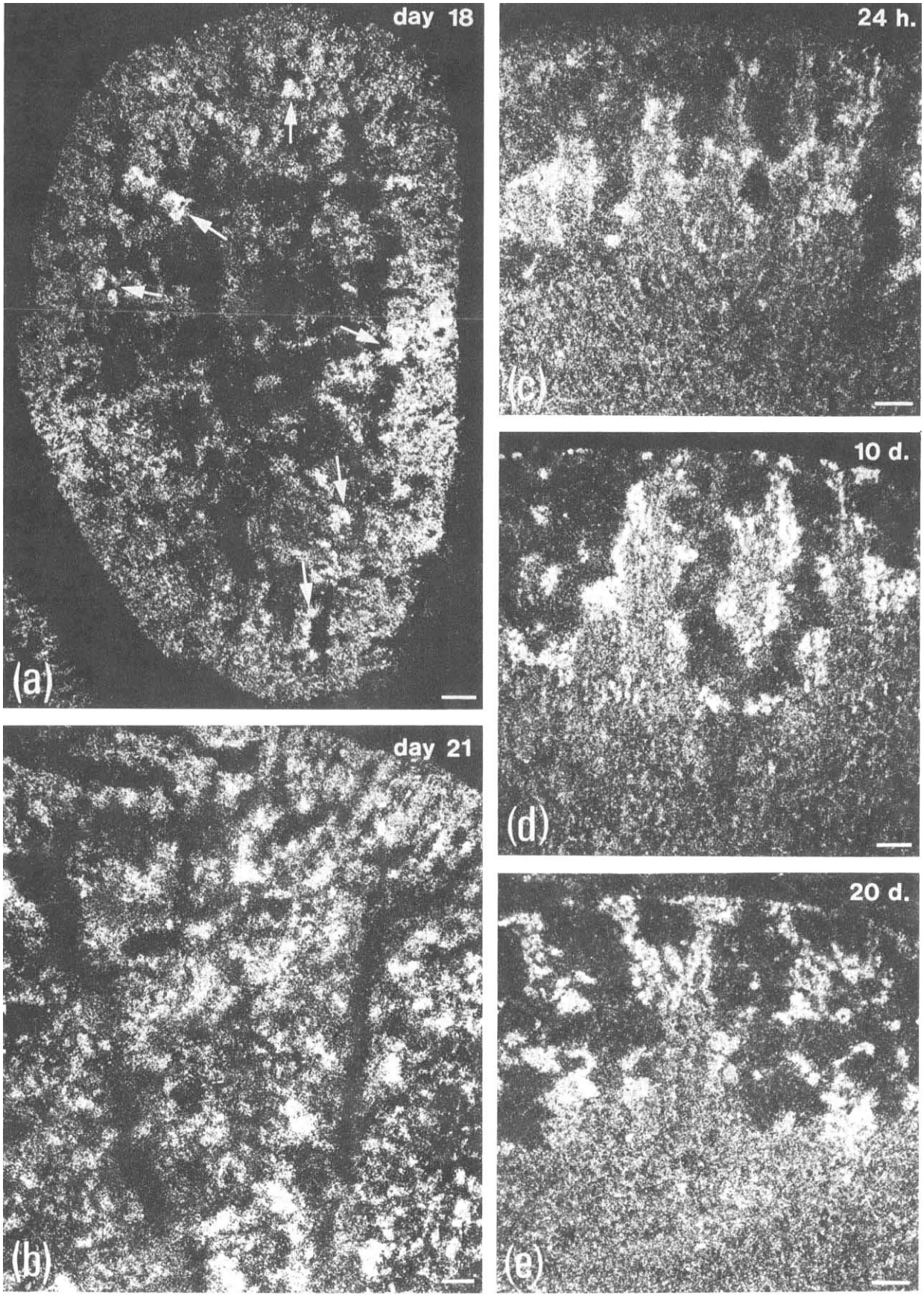
Total RNA from rat kidney was prepared, separated on agarose gels, and blotted onto Hybond N membranes as previously described for brain tissue [29]. Membranes were prehybridized in 50% formamide, 5 × SSPE, 0.1% SDS, 2 × Denhardt's, 100 μg/ml denatured salmon sperm DNA, and 200 μg/ml tRNA at 42°C for six hours after which 8 ng/ml of ^{32}P-labeled oligonucleotide probe were added and incubation was continued for an additional 18 to 20 hours. Membranes were washed with 2 × SSPE and 0.1% SDS (2 × 10 min at 42°C), 1 × SSPE and 0.1% SDS (1 × 20 min at 42°C), and 0.1 × SSPE and 0.1% SDS (1 × 10 min at room temperature) and apposed to film (Kodak X-Omat).

Results

The Northern blot analysis of total RNA extracted from whole adult rat kidneys and hybridized with either oligonucleotide probe showed a single transcript approximately 1.8 kb in length (Fig. 1). Using *in situ* hybridization, both oligonucleotide probes gave identical labeling patterns with equal intensity in both rat and human kidney. In film autoradiograms of adult (40 day old) rat kidney, strong COMT labeling was observed in the outer stripe of the outer medulla, with tubular rays radiating into the outer part of the cortex (Fig. 2). Weaker labeling was seen in the inner stripe (Fig. 2). With the exception of very weak labeling in the papilla, the inner medulla was devoid of labeling (Fig. 2). In emulsion-dipped and hematoxylin-counterstained sections, the labeling was demonstrated in segment S3 of proximal tubules, and in the medullary thick ascending limb (TAL) of loop of Henle (Fig. 3a-c). The different tubule segments were identified based on different staining properties with hematoxylin-eosin (proximal tubules more red that TAL cells) and morphological appearance (proximal tubules had higher epithelium and larger lumen as compared to TAL cells). During development, COMT mRNA was detected diffusely in the cortex as early as on prenatal day 18 (Fig. 2). At days 18 and 21, condensations of silver grains were present in the cortex (Fig. 4a,b). During day 18, and until eight days postnatally, the labeling was diffusely distributed in the cortex and medulla (Fig. 2); however, from 10 days the labeling was distributed in a more organized pattern, with the strongest labeling in the outer stripe and tubular rays radiating to the outer cortex (Fig. 2). At 20 days, the labeling in the inner stripe was apparent (Fig. 2). In emulsion-dipped sections, it was seen that the labeled tubular rays in the cortex were localized to the inner parts of the cortex at 24 hours within birth, and were developing towards the outer cortex at 10 days, to fully reach the outer surface of the cortex at 20 days (Fig. 4c-e). In the human kidney, COMT mRNA was seen in small tubular rays scattered in the entire cortex and in more densely organized in tubules of the medulla (Fig. 3d), where it in the latter could be identified in TAL cells (Fig. 3e-g). Intense COMT mRNA labeling was demonstrated in the rat ureter (Figs. 2 and 5), which in emulsion-dipped and counterstained sections was shown to be present in the transitional epithelium (Fig. 5a-d).

Discussion

The present study shows a nephron-specific distribution of COMT mRNA in renal tubule cells and shows for the first time that COMT also is produced in the ureter. In an earlier immunohistochemical study, COMT-like immunoreactivity was


day 18
(a)
day 21
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24 h.
(c)
10 d.
(d)
20 d.
(e)

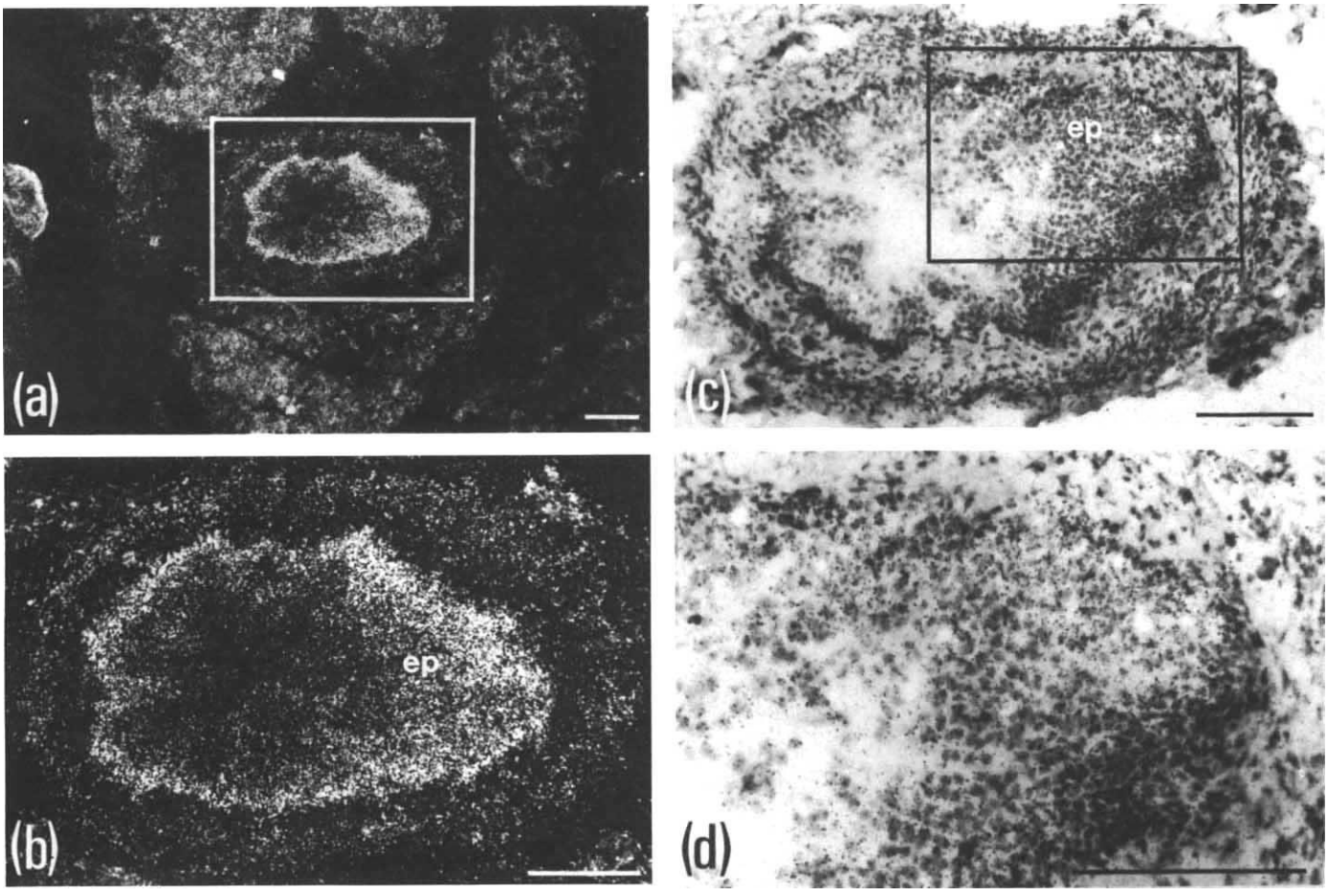

Fig. 5. *Dark- (a,b) and bright-field (c,d) photomicrographs of sections of rat ureter after* in situ *hybridization with an oligonucleotide probe to COMT mRNA.* Strong hybridization signal is demonstrated in the epithelium (ep) of the ureter (see higher magnifications in b and d as indicated by rectangles in a and c). Bars = 100 μm.

demonstrated along the luminal surfaces of the proximal and distal convoluted tubules and in collecting ducts [14], whereas in this study COMT mRNA was demonstrated in the straight portions of proximal and distal tubules and not in collecting ducts. The reason for the discrepancy between the immunohistochemical and *in situ* hybridization results is unclear, but potential cross reactivity of COMT antisera with other antigens may be considered. In our study specificity was evaluated by the use of two different oligonucleotide probes complementary to different parts of COMT mRNA, having similar guanosine/cytosine content and length, and the same specific activity. The probes gave identical results with equal intensity in labeling. Furthermore, the Northern blot analysis showed a transcript of 1.8 kb in length, in agreement with other results [24, 25].

The kidney is the main source of dopamine and dopamine metabolites in the urine [see 22]. Dopamine is produced in the kidney by conversion of L-DOPA via the enzyme aromatic L-amino acid decarboxylase (AADC) present in the cytoplasm of proximal tubule cells [30–35]. The catabolism of dopamine involves conversion of dopamine into DOPAC via the enzyme monoamine oxidase (MAO), and/or conversion of dopamine into 3-methoxytyramine (MTA) via COMT. DOPAC and MTA can be further converted into homovanillic acid by COMT and MAO, respectively. Homovanillic acid (HVA) is by far the major metabolite of dopamine excreted by the urine [see 36]. Only small fractions of dopamine metabolites seem to be excreted as a conjugate, and the small amounts of free urinary dopamine appears to be derived mainly from the decarboxylation of circulating DOPA [37]. The metabolism of norepinephrine also involves both MAO and COMT, and the main urinary

Fig. 4. *Dark-field photomicrographs of sections of rat kidney at prenatal day 18 (a) and 21 (b), within 24 hours of birth (c), and at postnatal day 10 (d) and 20 (e) after* in situ *hybridization with an oligonucleotide probe to COMT mRNA.* At day 18, weak labeling is observed in the cortex, with some condensations showing more intense labeling (arrows in a). At day 21, more condensations of labeled cells are present throughout the cortex (b). Within 24 hours of birth, labeling is shown in the inner cortex with particularly strongly labeled tubule cells radiating towards the outer part of the cortex (c). At 10 days postnatally, several of the intensely labeled tubule cells are reaching the surface of the outer cortex (d), and at 20 days many tubule cells are distributed in the outer cortex, reaching the renal capsule (e). Bars = 50 μm.

excretion product of norepinephrine is vanillyl mandelic acid (VMA). The demonstration of COMT synthesis in the kidney, coupled with studies on the effect of the COMT-inhibitor nitecapone on L-DOPA metabolism in healthy volunteers, indicates that COMT is of importance for renal dopamine metabolism [38]. Thus, when nitecapone was given concomitantly with L-DOPA/carbidopa, it decreased the urinary excretion of MTA and HVA [38].

Previous studies dealing with the inactivation and metabolism of catecholamines in the kidney have focused on MAO [39, 40]. In rat kidney slices loaded with L-DOPA, inhibition of MAO-A increased the accumulation of newly formed dopamine and reduced DOPAC formation, whereas inhibition of MAO-B did not affect dopamine formation, but decreased DOPAC levels [39, 40]. These results suggest that most of the MAO that is located inside tubular cells, in which dopamine synthesis takes place, is of the A-type [41]. In contrast, MAO-B, which is the dominating type in the cat renal cortex [42], may be located outside the compartment where dopamine synthesis occurs and would be responsible for the deamination of dopamine which leaks from renal tubule cells.

Selective presence of COMT mRNA in the S3 segment of proximal tubules and in TAL cells is interesting. Dopamine is produced in the S1 and S2 segments of proximal tubules [22]. The highest levels of excreted dopamine would consequently be expected to be encountered along the distal parts of proximal tubules, where inactivation of excessive dopamine should occur. In our study, the highest expression of COMT mRNA was demonstrated in the S3 segment of proximal tubules, whereas the expression in the TAL cells was lower, in agreement with this hypothesis.

Catecholamines play an important role for the regulation of renal sodium excretion [35]. It is possible that the level by which COMT is expressed in the kidney contributes to the modulation of renal sodium homeostasis. In fact, COMT mRNA levels appear to undergo dynamic changes after experimental manipulations. In this regard, we have recently shown that administration of 2% saline as drinking water results in changes in the COMT mRNA levels in the kidney (Meister et al, unpublished results).

The observation that COMT mRNA is present in the ureter suggests that the COMT produced is involved in inactivation of circulating catecholamines, rather than catecholamines released from noradrenergic nerve fibers in the smooth muscle layers of the ureter. Occurrence of COMT mRNA in the transitional epithelium suggests catabolism of urinary catecholamines even at a site located more distally to the nephron, and may serve to inactivate the excess of catecholamines that is not metabolized by renal tubule cells.

COMT mRNA was observed in the kidney of fetal and newborn rats. In these kidneys, COMT mRNA was diffusely located in the cortex. In the fetal kidneys, COMT mRNA was particularly well-expressed in the undifferentiated mesenchymal cells, which may suggest that catecholamines play a role for the formation of nephrons. Future studies are necessary to determine the role of COMT in the fetal kidney and factors of importance for the regulation of COMT mRNA in the mature kidney.

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